On the other hand, one may simply acquire, from various commercial sources, small molecule libraries that are believed to meet the basic criteria for useful drugs in an effort to "brute force" the identification of useful compounds. Screening of such libraries, including combinatorially generated libraries, is a rapid and efficient way to screen large number of related (and unrelated) compounds for activity. Combinatorial approaches also lend themselves to rapid evolution of potential drugs by the creation of second, third and fourth generation compounds modeled of active, but otherwise undesirable compounds.

Candidate compounds may include fragments or parts of naturally-occurring compounds, or may be found as active combinations of known compounds, which are otherwise inactive. It is proposed that compounds isolated from natural sources, such as plant sources, including leaves, bark, roots and fruit may be assayed as candidates for the presence of potentially useful pharmaceutical agents. It will be understood that the pharmaceutical agents to be screened could also be derived or synthesized from chemical compositions or man-made compounds. Thus, it is understood that the candidate substance identified by the present invention may be saponins, cholesterol, other fatty acids or other small molecules or any other compounds that may be designed through rational drug design starting from known target compounds.

B. *In vitro* Assays

A quick, inexpensive and easy assay to run is an *in vitro* assay. Such assays generally use isolated molecules, can be run quickly and in large numbers, thereby increasing the amount of information obtainable in a short period of time. A variety of vessels may be used to run the assays, including test tubes, plates, dishes and other surfaces such as dipsticks or beads.

One example of a cell free assay is a binding assay. While not directly addressing function, the ability of a candidate substance to bind to a target molecule in a specific fashion is strong evidence of a related biological effect. For example, binding of a

molecule to a target may, in and of itself, be inhibitory, due to steric, allosteric or charge-charge interactions. The target may be either free in solution, fixed to a support, expressed in or on the surface of a cell. Either the target or the compound may be labeled, thereby permitting determining of binding. Usually, the target will be the labeled species, decreasing the chance that the labeling will interfere with or enhance binding. Competitive binding formats can be performed in which one of the agents is labeled, and one may measure the amount of free label versus bound label to determine the effect on binding.

Another example of an *in vitro* assay, may include incubating or bathing an isolated tissue, *e.g.*, adipose or skeletal muscle, in a solution containing the candidate substance and measuring parameters that are well known in the art to change in response to insulin or glucose. Physiological and molecular analysis may be performed. For example, Northern or Western analysis may be preformed to measure changes in mRNA or protein levels.

C. *In cyto* Assays

The present invention also contemplates the screening of compounds for their ability to modulate metabolic activity in cells. Primary cell culture may be utilized to study various tissue responses in control and diabetic animal models. For example, hepatocyte primary cell cultures may be developed from livers of control and diabetic animals using standard techniques well known and used in the art of tissue culturing. Once the cell culture is established, these cells may then be incubated with various candidate substances and various parameters may be measured, *e.g.*, biochemical or molecular. Biochemical assays may include, for example, enzyme analysis. Molecular analysis may be preformed, for example, looking at protein expression, mRNA expression and others.

D. *In vivo* Assays

In vivo assays involve the use of various animal models, including transgenic animals that have been engineered to have specific defects. In the present invention, the *ob/ob* mouse model is preferred. It is also contemplated that other diabetic transgenic mouse models may be used, for example, Jackson Laboratories has an extended inventory of transgenic diabetic mice, *e.g., db/db* mouse model. In addition to transgenic mice, chemically induced diabetic mouse and rat models are also available and they have also been contemplated and are within the scope of the present invention. These models include, but are not limited to, alloxan-diabetes mice and streptozotocin-induced diabetic rats.

Due to their size, ease of handling, and information on their physiology and genetic make-up, mice are a preferred embodiment, especially for transgenics. However, other animals are suitable as well, including rats, rabbits, hamsters, guinea pigs, gerbils, woodchucks, cats, dogs, sheep, goats, pigs, cows, horses and monkeys (including chimps, gibbons and baboons).

In specific embodiments, one or more candidate substances are administered to an animal, and the ability of the candidate substance(s) to alter one or more characteristics, as compared to a similar animal not treated with the candidate substance(s), identifies an active compound. The characteristics may be any of those discussed above with regard to the function alteration of blood glucose, plasma cholesterol, glucose utilization, energy consumption, food intake, body temperature and other metabolic parameter.

Treatment of these animals with test compounds will involve the administration of the compound, in an appropriate form, to the animal. Administration will be by any route that could be utilized for clinical or non-clinical purposes, including but not limited to oral, nasal, buccal, or even topical. Alternatively, administration may be by intratracheal instillation, bronchial instillation, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection. Specifically contemplated routes are systemic